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MINTZ LEVIN COHN FERRIS GLOVSKY & POPEO

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BOSTON, MA 02111

EXAMINER

THOMAS, DAVID C

ART UNIT

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DELIVERY MODE

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PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary**Application No.**

10/767,899

Applicant(s)

BERKA ET AL.

Examiner

DAVID C. THOMAS

Art Unit

1637

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --
Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 01 April 2008.
- 2a) ☒ This action is **FINAL**. 2b) ☐ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) ☒ Claim(s) 1-3, 6-29 and 32-44 is/are pending in the application.
- 4a) Of the above claim(s) 16-21, 23 and 35-44 is/are withdrawn from consideration.
- 5) ☐ Claim(s) _____ is/are allowed.
- 6) ☒ Claim(s) 1-3, 6-15, 22, 24-29 and 32-34 is/are rejected.
- 7) ☐ Claim(s) _____ is/are objected to.
- 8) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on _____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All b) ☐ Some * c) ☐ None of:
1. ☐ Certified copies of the priority documents have been received.
 2. ☐ Certified copies of the priority documents have been received in Application No. _____.
 3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- 1) ☒ Notice of References Cited (PTO-892)
- 2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
- 3) ☐ Information Disclosure Statement(s) (PTO/SB/08)
Paper No(s)/Mail Date _____
- 4) ☐ Interview Summary (PTO-413)
Paper No(s)/Mail Date _____
- 5) ☐ Notice of Informal Patent Application
- 6) ☐ Other: _____

DETAILED ACTION

1. Applicant's response filed April 1, 2008 is acknowledged. Claims 1-3, 6-14, 22, 24, 25, 32 and 33 (original or previously presented) will be examined on the merits. Claims 16-21, 23 and 34-44 were previously withdrawn and claims 4, 5, 30 and 31 were previously canceled.

Claim Rejections - 35 USC § 103

2. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

3. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

4. Claims 1-3, 6-14, 22, 24, 25, 32 and 33 are rejected under 35 U.S.C. 103(a) as being unpatentable over Griffiths (U.S. Patent No. 2002/0119459) in view of Wangh et

al. (U.S. Patent Pub. No. 2004/0053254) and further in view of Strizhkov et al. (BioTechniques (2000) 29:844-857).

Griffiths teaches a method for amplifying one or more nucleic acids onto a bead (DNA or RNA in microcapsules can be amplified by various methods, paragraph 23, lines 5-10 and paragraph 98, lines 1-22) comprising the steps of:

(a) forming a water-in-oil emulsion to create a plurality of aqueous microreactors (emulsion has a water phase containing the biomolecules and an inert hydrophobic phase of oil, paragraph 91, lines 14) wherein at least one of the microreactors comprises one single stranded nucleic acid template (microcapsules contain, on average, one or fewer genetic elements each, including RNA, paragraph 81, lines 1-12, paragraph 98, lines 1-3 and paragraph 125, lines 1-6), and an amplification reaction solution comprising a population comprising a plurality of molecules of the first primer species and a plurality of molecules of a second primer species and reagents necessary to perform nucleic acid amplification wherein the first primer species is capable of binding to the single stranded nucleic acid template and the second primer species is capable of binding to a complementary strand of the single stranded nucleic acid template (nucleoside triphosphates and other necessary reagents are required in the microcapsules for amplification reactions such as PCR or those involving transcription steps and requiring two primers for binding the target and complementary strands, paragraph 98, lines 1-23, paragraph 100, lines 1-6 and paragraph 101, lines 1-8);

(b) amplifying the single stranded nucleic acid template and the complementary strand to the template in the amplification reaction solution to form a population of

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amplified copies of the single stranded template nucleic acid (DNA or RNA in microcapsules can be amplified by various methods including PCR using one of the primers as a biotinylated primer, paragraph 98, lines 1-23, paragraph 110, lines 1-5 and paragraph 217, lines 1-11); and

(c) binding a plurality of the amplified copies of the single stranded template nucleic acid to the bead in the microreactor (nucleic acids amplified using biotinylated primers can be captured onto microbeads coated with avidin, paragraph 110, lines 1-5 and paragraph 111, lines 1-10);

(d) breaking the aqueous microreactors to release at least one of the nucleic acid bound beads and the amplification reaction solution comprising unbound amplification products (microcapsules containing genetic elements such as nucleic acid templates bound to beads, along with amplification reaction mixtures, may be broken for purposes of pooling the templates comprising the genetic elements, paragraph 168, lines 1-6); and

(e) recovering the nucleic acid bound beads (reagents may be added which bind to or react with the bead-bound nucleic acids and their products, to enrich for the genetic elements with modified optical properties, paragraphs 168-171; product-bound nucleic acids can also be reacted with antibodies in the microcapsules before pooling of the tagged nucleic acids by breaking the microcapsules, paragraph 172, lines 1-16).

With regard to claim 2, Griffiths teaches a method for amplifying one or more nucleic acids wherein a majority of the microreactors include a single nucleic acid

(microcapsules contain, on average, one or fewer genetic elements each, paragraph 81, lines 1-12).

With regard to claim 3, Griffiths teaches a method for amplifying one or more nucleic acids wherein said amplification reaction solution is a polymerase chain reaction solution further comprising nucleotide triphosphates, a thermostable polymerase, and a buffer compatible with polymerase chain reaction conditions (NTPs and other necessary reagents are required in the microcapsules for enzymatic reactions, paragraph 100, lines 1-6 and 101, lines 1-8; PCR can be used if the emulsions are thermostable, paragraph 98, lines 18-23).

With regard to claims 6 and 7, Griffiths teaches a method for amplifying one or more nucleic acids wherein said emulsion additionally contains emulsion stabilizers (emulsions may be stabilized by addition of one or more surface-active agents or surfactants such as Span 80, paragraph 92, lines 1-11).

With regard to claims 8 and 9, Griffiths teaches a method for amplifying one or more nucleic acids wherein said emulsion is heat stable to 95°C (emulsions can be made to work under PCR conditions, paragraph 98, lines 18-23).

With regard to claim 10, Griffiths teaches a method for amplifying one or more nucleic acids wherein amplification is carried out by a method of transcription-based amplification (transcription of the DNA to amplify the nucleic acid copies, paragraph 98, lines 1-12).

With regard to claim 11, Griffiths teaches a method for amplifying one or more nucleic acids wherein the emulsion is formed by the dropwise addition of the nucleic

acid templates, beads, and amplification reaction solution into an oil (droplets of microscopic or colloidal size of aqueous phase are added to hydrophobic oil phase, paragraph 90, lines 1-6 and paragraph 91, lines 1-9).

With regard to claims 12 and 13, Griffiths teaches a method for amplifying one or more nucleic acids performed with at least 50,000 nucleic acids (reactions that contain on average one nucleic molecule and one bead (paragraph 23, lines 5-14), contain about 10^9 beads in suspension and therefore as many as 10^9 copies of the nucleic acid, paragraph 300, lines 5-13, paragraph 301, lines 1-4, and paragraph 302, lines 1-2).

With regard to claim 14, Griffiths teaches a method of amplifying nucleic acid in a microreactor such as a water-in-oil emulsion wherein the emulsion droplets range from 0.1 to 10 μm in diameter (paragraph 103, lines 1-6).

With regard to claim 22, Griffiths teaches a method for amplifying a nucleic acid onto a bead comprising the steps of:

(a) providing one single stranded nucleic acid template to be amplified (one genetic element such as RNA is provided in each microcapsule, paragraph 125, lines 1-6);

(b) providing a solid support material comprising a generally spherical bead having a diameter about 10 to about 80 μm , wherein the bead comprises a plurality of molecules of a first population of a first primer species disposed thereon capable of binding to the nucleic acid template (paramagnetic beads containing avidin coating in order to bind nucleic acid are about 5 μm in diameter, paragraph 111, lines 4-10; one of

the PCR primers can be biotinylated and therefore capable of binding to the avidin-coated beads, paragraph 110, lines 1-5);

(c) mixing the nucleic acid template and the bead in an amplification reaction solution comprising a plurality of molecules of a second population of the first primer species (such as biotinylated primer at allow binding of the products to avidin-coated beads, paragraph 110, lines 1-5), a second primer species and reagents necessary to perform a nucleic acid amplification reaction in a water-in-oil emulsion, wherein the first primer species is capable of binding to the single stranded nucleic acid template and the second primer species is capable of binding to a complementary strand of the single stranded nucleic acid template (nucleoside triphosphates and other necessary reagents are required in the microcapsules for amplification reactions such as PCR or those involving transcription steps and requiring two primers for binding the target and complementary strands, paragraph 98, lines 1-23, paragraph 100, lines 1-6 and paragraph 101, lines 1-8);

(d) amplifying the single stranded nucleic acid template and the complementary strand of the single stranded nucleic acid template in the amplification reaction solution using the second population of the first primer species and the second primer species to form a population of amplified copies of the single stranded template nucleic acid (DNA or RNA in microcapsules can be amplified by various methods including PCR using one of the primers as a biotinylated primer, paragraph 98, lines 1-23, paragraph 110, lines 1-5 and paragraph 217, lines 1-11); and

(e) binding a plurality of the amplified copies of the single stranded template nucleic acid to the bead in the microreactor (nucleic acids amplified using biotinylated primers can be captured onto microbeads coated with avidin, paragraph 110, lines 1-5 and paragraph 111, lines 1-10); and

(f) recovering the nucleic acid bound beads (reagents may be added which bind to or react with the bead-bound nucleic acids and their products, to enrich for the genetic elements with modified optical properties, paragraphs 168-171; product-bound nucleic acids can also be reacted with antibodies in the microcapsules before pooling of the tagged nucleic acids by breaking the microcapsules, paragraph 172, lines 1-16).

With regard to claim 24, Griffiths teaches a method for amplifying one or more nucleic acids further comprising the step of enriching for beads which bind amplified copies of the nucleic acid away from beads to which no nucleic acid is bound, the enrichment step consisting of cell sorting (beads which contain fluorescent signals due to amplification and subsequent binding of groups involved in generating fluorescent signal can be sorted by flow cytometry, paragraph 202, lines 1-14).

With regard to claim 25, Griffiths teaches a method for amplifying one or more nucleic acids wherein the enrichment step is performed by affinity purification with magnetic beads that bind nucleic acid (enrichment steps can be performed using magnetic beads and a magnet, paragraph 239, lines 34-45).

With regard to claim 32, Griffiths teaches a method for amplifying one or more nucleic acids further comprising the steps of:

separating the template carrying beads and magnetic bead (such as by flow sorting, paragraph 202, lines 1-14; and

removing the magnetic beads with a magnetic field (enrichment steps can be performed using magnetic beads and a magnet, paragraph 239, lines 34-45).

With regard to claim 33, Griffiths teaches a method for amplifying one or more nucleic acids wherein the separating is achieved by incubating the template carrying beads and the magnetic beads in a solution with a basic pH (solution containing beads were suspended in buffer containing 5 mM Tris 7.4 prior to transcription, translation and flow cytometry and incubated at 43°C, paragraph 238, lines 1-10).

Griffiths does not teach methods of amplifying a nucleic acid wherein two populations of a first primer are used, one bound to a solid surface such as a bead, and the other in an amplification reaction solution. Griffiths also does not teach methods of amplifying one or more nucleic acids wherein a concentration of the second primer species is substantially greater than that of the first primer species in the reaction solution and wherein substantially all of the molecules of the first primer species in the reaction solution are depleted. Griffiths also does not teach a method wherein a plurality of copies of the single stranded template nucleic acid are bound to the first population of the first primer species on the bead and wherein a bead-bound complementary strand is extended from the first primer species. Griffiths also does not teach microreactors having an average size of 50 to 250 μm in diameter.

Wangh teaches a method of performing non-symmetric PCR using one amplification primer that is present at a concentration at least five times greater than the

other primer such that the limiting primer can be used to exhaustion (paragraph 25, lines 1-22 and paragraph 30, lines 1-7). Wangh also teaches a method wherein one of the primers, the limiting one or the primer in excess, is fixed to a solid matrix such as a bead (paragraph 181, lines 1-7). Finally, Wangh teaches a multiplexed method for non-symmetric PCR (paragraph 70, lines 1-12).

Strizhkov teaches a method of amplifying a target DNA sequence on a microarray and in the solution phase using target-specific primers for PCR (see Abstract), wherein the forward PCR primer is immobilized to a microarray gel pad, while the same forward primer is also in the solution phase to allow diffusion of amplification products and reagents between the solution phase and the gel pad and also allow amplification to proceed initially in solution, and in subsequent rounds on the gel pad using the immobilized primer, the products of the solid-phase reaction being used for detection (see Abstract and p. 848, column 1, line 46 to column 3, line 22, p. 856, column 2, line 32 to column 3, line 24 and also Figure 1). The unlabeled forward and labeled reverse primers are present in solution at 1-10 pmol each (p. 845, column 3, lines 49-52). This system can also be performed in a multiplex format (p. 856, column 3, lines 24- 33).

It would have been prima facie obvious to one having ordinary skill in the art at the time the invention was made to combine the method of Griffiths for amplifying nucleic acids in a microcapsule such as a water-in-oil emulsion on the surface of a bead with the method of Wangh for non-symmetric PCR using primers at unequal

concentrations since this method, which causes one of the primers to be used to exhaustion, is highly suited for amplifications that utilize small reaction volumes and very low copy numbers of target sequences (Wangh, paragraph 32, lines 1-8). It would also have been prima facie obvious to utilize two populations of a first primer, one attached to a solid surface and one in solution, since the primer in solution can perform the initial rounds of amplification on the target nucleic acid, while subsequent rounds of amplification of the resulting extension products can be further amplified in the solid-phase for detection using the immobilized forward primer and the labeled reverse primer present in solution (Strizhkov, p. 848, column 2, line 17 to column 3, line 7).

Furthermore, an ordinary practitioner would have been motivated to use a non-symmetric PCR assay as taught by Wangh using one of the primers affixed to a bead surface since the assay not only is highly suited for detecting low levels of target sequence, but the extension products will remain attached to the bead or other solid surface (Wangh, paragraph 181, lines 1-7), thus allowing for easy detection of the products containing incorporated labels when used in solid-phase systems such as those taught by Strizhkov (Strizhkov, p. 848, column 3, lines 8-15 and Figure 1). The methods of Strizhkov provide for an efficient two-phase amplification since the forward primer in solution will be more efficient for amplification of the larger starting DNA template during initial rounds of the reaction, while the smaller extension products can more easily diffuse over a solid-phase surface to amplify the products using the immobilized forward primer and the labeled reverse primer present in solution during the detection phase (Strizhkov, p. 848, column 2, line 17 to column 3, line 7). Furthermore,

the non-asymmetric assay of Wangh is easily adaptable to performing in microcapsules such as water-in-oil emulsion droplets since such microcapsules are sufficiently large to accommodate any conditions or reaction requirements for amplification reactions such as PCR (Griffiths, paragraph 98, lines 18-23 and paragraph 100, lines 1-6). Moreover, the use of microcapsules allows reactions to be compartmentalized to separate different target sequences from each other, yet targets at very low copy number can still be readily amplified (Griffiths, paragraph 81, lines 1-16).

It would also have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to use emulsion droplets of larger sizes such as in the range of 50 μm as used by the applicant or in the range of 10 μm as used by Griffiths since these differences in emulsion droplet size would not be expected to greatly alter the conditions for amplification. Though the effective concentration of a single template DNA would be lower in the larger droplets, this would be offset by the larger absolute amounts amplification reagents such as nucleotides and primers. This is consistent with the Federal Circuit decision in In re Peterson, 65 USPQ2d 1379, 1382 (Fed. Cir. 2003) "We have also held that a *prima facie* case of obviousness exists when the claimed range and the prior art range do not overlap but are close enough such that one skilled in the art would have expected them to have the same properties." Thus, an ordinary practitioner would have recognized that the droplet size could be adjusted to maximize the desired results. As noted in *In re Aller*, 105 USPQ 233 at 235,

More particularly, where the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation.

Routine optimization is not considered inventive and no evidence has been presented that the selection of droplet size was other than routine, that the products resulting from the optimization have any unexpected properties, or that the results should be considered unexpected in any way as compared to the closest prior art. As noted, a skilled artisan would expect droplet sizes of 10-50 μm to have nearly identical properties in the amplification of nucleic acids. Thus, an ordinary practitioner would have recognized that the results could be adjusted to maximize the desired results.

5. Claims 15 and 26-29 are rejected under 35 U.S.C. 103(a) as being unpatentable over Griffiths (U.S. Patent No. 2002/0119459) in view of Wangh et al. (U.S. Patent Pub. No. 2004/0053254) and further in view of Strizhkov et al. (BioTechniques (2000) 29:844-857) and further in view of Jurinke et al. (U.S. Patent No. 6,303,309).

Griffiths, Wangh and Strizhkov together teach the limitations of claims 1-3, 6-14, 22, 24, 25, 32 and 33 as discussed above.

Neither Griffiths nor Wangh nor Strizhkov teach a method for amplifying one or more nucleic acids wherein more than 10,000 or at least 1,000,000 amplification copies of each target nucleic acid molecule are bound to each bead. Neither Griffiths nor Wangh nor Strizhkov teach a method for amplifying one or more nucleic acids wherein the beads are sepharose beads.

Jurinke teaches a method of purification of biotin-labeled PCR products by complexing the products to a solid support containing a biotin-binding compound such as streptavidin immobilized on the surface, including agarose, sepharose, or magnetic

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beads (column 8, lines 49-60). Jurinke also teaches immobilization of 100 pmol biotinylated oligodeoxynucleotide to 50 μ l (~40 million) streptavidin-coated magnetic beads (column 8, 12, lines 13-16), which represents about 1 million molecules bound per bead.

It would have been *prima facie* obvious to one having ordinary skill in the art at the time the invention was made to combine the methods of Griffiths, Wangh and Strizhkov for amplifying nucleic acids on a bead within a microcapsule such as a water-in-oil emulsion using a non-symmetric PCR with that of Jurinke for purification of PCR products using solid-supports such as magnetic or sepharose beads since the use of such beads, because of the stability of the biotin-streptavidin complex, allows further purification and extensive washing to remove all excessive reaction components prior to final recovery of the final PCR product (Jurinke, column 8, lines 61-67). Thus, an ordinary practitioner would have been motivated to use magnetic or sepharose beads as taught by Jurinke for binding and purifying PCR or other amplification products generated in a microreactor since these beads have a large capacity and high affinity for such products, especially when using highly stable binding pairs such as biotin and streptavidin to form complexes of the amplification products on the beads.

6. Claim 34 is rejected under 35 U.S.C. 103(a) as being unpatentable over Griffiths (U.S. Patent No. 2002/0119459) in view of Wangh et al. (U.S. Patent Pub. No. 2004/0053254) and further in view of Strizhkov et al. (BioTechniques (2000) 29:844-857) and further in view of Nakano et al. (J. Biotech. (2003) 102:117-124).

With regard to claim 34, Griffiths teaches a method for producing a clonal population of nucleic acids, comprising:

(a) providing a single stranded nucleic acid template to be amplified (one genetic element, such as RNA, is provided in each microcapsule, paragraph 81, lines 1-12, paragraph 98, lines 1-3 and paragraph 125, lines 1-6);

(b) mixing the single stranded nucleic acid template and the beads in an amplification reaction solution that comprises a second population of a plurality of molecules of the first primer species (such as a biotinylated primer to allow binding of the products to avidin-coated beads, paragraph 110, lines 1-5), a plurality of molecules of a second primer species and reagents necessary to amplify the nucleic acid template, wherein the first primer species is capable of binding to the single stranded nucleic acid template and the second primer species is capable of binding to a complementary strand of the single stranded nucleic acid template (nucleoside triphosphates and other necessary reagents are required in the microcapsules for amplification reactions such as PCR or those involving transcription steps and requiring two primers for binding the target and complementary strands, paragraph 98, lines 1-23, paragraph 100, lines 1-6 and paragraph 101, lines 1-8); and

(c) forming an emulsion to create a plurality of microreactors (emulsion has a water phase containing the biomolecules and an inert hydrophobic phase of oil, paragraph 91, lines 14) comprising the nucleic acid template, beads, and the amplification reaction solution, wherein at least one of the microreactors comprises a single nucleic acid template (microcapsules contain, on average, one or fewer genetic

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elements each, paragraph 81, lines 1-12 and paragraph 125, lines 1-6) and a single bead encapsulated in the amplification reaction solution (nucleic acid may be linked to one or more magnetic beads, paragraph 23, lines 5-10), wherein the microreactors are contained in the same vessel (such as a microtiter plate or microfuge tube, paragraph 290, lines 1-5 and paragraph 291, lines 1-9).

Griffiths does not teach methods of amplifying a plurality of nucleic acid templates from 50-800 bp in length wherein two populations of a first primer are used, one bound to a solid surface such as a bead, and the other in an amplification reaction solution. Griffiths also does not teach methods of amplifying one or more nucleic acids wherein a concentration of the second primer species is substantially greater than that of the first primer species in the reaction solution and wherein substantially all of the molecules of the first primer species in the reaction solution are depleted. Griffiths also does not teach a method wherein a plurality of copies of the single stranded template nucleic acid are bound to the first population of the first primer species on the bead. Griffiths also does not teach microreactors having an average size of 50 to 250 μm in diameter.

Wangh teaches a method of performing non-symmetric PCR using one amplification primer that is present at a concentration at least five times greater than the other primer such that the limiting primer can be used to exhaustion (paragraph 25, lines 1-22 and paragraph 30, lines 1-7). Wangh also teaches a method wherein one of the primers, the limiting one or the primer in excess, is fixed to a solid matrix such as a bead (paragraph 181, lines 1-7).

Strizhkov teaches a method of amplifying a target DNA sequence on a microarray and in the solution phase using target-specific primers for PCR (see Abstract), wherein the forward PCR primer is immobilized to a microarray gel pad, while the same forward primer is also in the solution phase to allow diffusion of amplification products and reagents between the solution phase and the gel pad and also allow amplification to proceed initially in solution, and in subsequent rounds on the gel pad using the immobilized primer, the products of the solid-phase reaction being used for detection (see Abstract and p. 848, column 1, line 46 to column 3, line 22, p. 856, column 2, line 32 to column 3, line 24 and also Figure 1). The unlabeled forward and labeled reverse primers are present in solution at 1-10 pmol each (p. 845, column 3, lines 49-52). This system can also be performed in a multiplex format (p. 856, column 3, lines 24- 33).

Nakano teaches a method of PCR in water-in-oil emulsions using a plurality of templates of 528 and 512 bp in size (p. 118, column 2, line 39 to p. 119, column 1, line 8).

It would have been *prima facie* obvious to one having ordinary skill in the art at the time the invention was made to combine the methods of Griffiths, Wangh and Strizhkov for amplifying nucleic acids on a bead within a microcapsule such as a water-in-oil emulsion using a non-symmetric PCR with that of Nakano since Nakano also teaches a method for amplifying multiple nucleic acids in water-in-oil emulsions that is easily adaptable to the methods of Griffiths, Wangh and Strizhkov using a bead to bind amplification products in the emulsion. Thus, an ordinary practitioner would have been

motivated to use the combined system of Griffiths, Wangh and Strizhkov for amplifying multiple nucleic acid targets of different sizes in an emulsion containing a bead using a non-symmetric PCR process since the products can easily be purified simultaneously on the same bead and later separated by sizing methods or simply analyzed by gel electrophoresis (Nakano, see Figure 2).

Response to Arguments

7. Applicant's arguments filed April 1, 2008 have been fully considered but they are not persuasive.

Applicant argues that the 35 USC § 103(a) rejections of claims 1-3, 6-14, 22, 24, 25, 32 and 33 as being unpatentable over Griffiths (U.S. Patent Pub. No. 2002/0119459) in view of Wangh et al. (U.S. Patent Pub. No. 2004/0053254) and further in view of Yu et al. (U.S. Patent Pub. No. 2001/0036632) should be withdrawn since the combination of the references do not teach all the limitations of the claims. In particular, Applicant argues none of the cited references teach or suggest a method of amplifying nucleic acids wherein a first primer is present both in solution and in an immobilized form on a solid support, with a second primer in solution that is present at a much higher concentration than the first primer in solution such that the first primer is depleted to allow amplification to occur only on the solid-phase support. The Examiner had alleged that Yu teaches a method using both solid-phase and solution-phase primers and that these methods could be combined with the methods of Wangh for non-symmetric amplification to meet this limitation of the claims. The Examiner agrees that

neither Yu nor Wangh teach two distinct populations of a first primer, one in solution and one immobilized on a solid support. Therefore, the 103(a) rejection over Griffiths in view of Wangh and further in view of Yu is withdrawn.

However, upon further searching, a new reference was found (Strizhkov et al., *BioTechniques* (2000) 29:844-857) that teaches methods of amplification on a microarray in which a first primer may be linked both to a solid support and present in solution wherein products of amplification generated in solution using a second primer bind back to the support by annealing to the first primer, which can then initiate additional rounds of amplification to yield products primarily attached to the solid support (Strizhkov, p. 848, column 2, line 17 to column 3, line 7). It is obvious that the methods of Wangh using unequal concentrations of solution-based primers can be combined with the solid phase methods of Strizhkov using a forward primer present in both the solid and solution phases, with the second primer in solution, which is labeled in the methods of Strizhkov, chosen as the more abundant primer species in solution. Copies initiated by the second primer will readily anneal to the bound first primer to initiate additional rounds of amplification and accumulation of products bound to the support as the first primer in solution is depleted, resulting in production of high levels of labeled PCR products bound to the solid support. Therefore, claims 1-3, 6-14, 22, 24, 25, 32 and 33 are now rejected under 35 USC § 103(a) over Griffiths in view of Wangh and further in view of Strizhkov.

Applicant then argues that the 35 USC § 103(a) rejection of claims 15 and 26-29 over Griffiths in view of Wangh and further in view of Yu and further in view of Jurinke et

al. (U.S. Patent No. 6,303,309) should be withdrawn since the combination of the references do not teach all the limitations of the claims. In particular, Applicant argues that Jurinke fails to cure the deficiencies of Griffiths, Wangh and Yu for teaching methods of amplifying nucleic acids on a solid phase support in an asymmetric manner, particularly with regard to the limitation requiring two distinct first primer populations. As discussed above, claims 1-3, 6-14, 22, 24, 25, 32 and 33 are now rejected under 35 USC § 103(a) over Griffiths in view of Wangh and further in view of Strizhkov. Therefore, claims 15 and 26-29 are now rejected under 35 USC § 103(a) over Griffiths in view of Wangh and further in view of Strizhkov and further in view of Jurinke since this rejection is not argued separately with regard to Jurinke.

Finally, Applicant then argues that the 35 USC § 103(a) rejection of claim 34 over Griffiths in view of Wangh and further in view of Yu and further in view of Nakano et al. (J. Biotech. (2003) 102:117-124) should be withdrawn since the combination of the references do not teach all the limitations of the claims. In particular, Applicant argues that Nakano fails to cure the deficiencies of Griffiths, Wangh and Yu for teaching methods of amplifying nucleic acids on a solid phase support in an asymmetric manner, particularly with regard to the limitation requiring two distinct first primer populations. As discussed above, claims 1-3, 6-14, 22, 24, 25, 32 and 33 are now rejected under 35 USC § 103(a) over Griffiths in view of Wangh and further in view of Strizhkov. Therefore, claim 34 is now rejected under 35 USC § 103(a) over Griffiths in view of Wangh and further in view of Strizhkov and further in view of Nakano since this rejection is not argued separately with regard to Nakano.

Conclusion

8. Claims 1-3, 6-15, 22, 24-29 and 32-34 are rejected.

Correspondence

9. Any inquiry concerning this communication or earlier communications from the examiner should be directed to David C. Thomas whose telephone number is 571-272-3320 and whose fax number is 571-273-3320. The examiner can normally be reached on 5 days, 9-5:30.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on 571-272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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/David C Thomas/
Examiner, Art Unit 1637

/Kenneth R Horlick/
Primary Examiner, Art Unit 1637